PATENT
Docket No. 295002005025

CERTIFICATE OF HAND DELIVERY

I hereby certify that this correspondence is being hand filed with the United States Patent and Trademark Office in Washington, D.C. on October 18, 1999.

R. Lynn Cruz

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of:

Irving BOIME et al.

Serial No.: 08/918,288

Filing Date: August 25, 1997

For: SINGLE CHAIN FORMS OF THE GLYCOPROTEIN HORMONE QUARTET

Examiner: L. Spector

Group Art Unit: 1646

DECLARATION OF DR. AARON HSUEH PURSUANT TO 37 C.F.R. § 1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

I, Aaron Hsueh, declare as follows:

1. I have been engaged in research on proteins involved in reproduction, including the glycoprotein hormones and related proteins for a number of years. I am currently a professor of _____ at Stanford University. A copy of my curriculum vitae is enclosed as Exhibit A.

2. I attempted to prepare a single-chain form of the protein inhibin (which regulates FSH secretion). Inhibins are heterodimeric proteins with alpha and beta subunits covalently linked by disulfide bonds. The subunits are initially produced as pro proteins -- i.e., as precursors containing pro sequences fused to the N-terminus of the mature subunit in each case.

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3. In my initial attempt to synthesize a single-chain form of inhibin, I prepared a nucleotide sequence wherein the 3' end of the nucleotide sequence encoding the mature β-subunit was fused to the 5' end of the mature α-subunit which did not contain the pro domain -- i.e., the nucleotide sequence prepared encoded a single-chain form containing mature β-subunit linked to the N-terminus of the mature α-subunit. This construct was placed into a plasmid under control of the CMV promoter and the resulting expression plasmid transfected into CHO cells. The cells were cultured for expression, but no secretion of the single-chain protein occurred. Intracellularly produced protein did react with antibodies prepared against the α-subunit of inhibin.

4. In lieu of the construct described in the preceding paragraph, an additional construct was made which linked the 3' terminus of the nucleotide sequence encoding the β-subunit to the nucleotide sequence encoding the N-terminus of the α-subunit. Thus the α-subunit pro sequence was included. A single-chain inhibin was secreted which was immunoreactive with antibodies against the α-subunit. However, the secreted protein was apparently not biologically active in mimicking inhibin activity.

5. I conclude from the results of this experiment that it is not straightforward to construct a biologically active single-chain form of heterodimers generally. The need particularly to design the construct so that secretion is effected is shown by these results, as well as the difficulty in producing biologically active product.

6. An abstract describing these results is attached as Exhibit B.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Executed at Palo Alto, California, on August 27 1999, by

Aaron Hsueh

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FF

Principal Investigator/Program Director (Last, first, middle): HSUEH, AARON J.W.

BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

NAME	POSITION TITLE
Aaron J.W. Hsueh, Ph.D.	Professor

EDUCATION *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
National Taiwan University, Taiwan	B.S.	1969	Zoology
Purdue University, Indiana	M.S.	1972	Endocrinology
Baylor College of Medicine, Texas	Ph.D.	1975	Cell Biology
Reproductive Research Branch, NICHD	PostDoc	1976	Reproduction

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

1976-1981 Assistant Adjunct Professor, Reproductive Medicine, University of California, San Diego
1981-1984 Associate Professor, Reproductive Medicine, University of California, San Diego
1984-1990 Professor, Reproductive Medicine, University of California, San Diego
1991-Present Professor, Reproductive Biology, Stanford University

MEMBER: 1984-1987 Endocrinology Study Section, NIH
1989-1993 Population Research Committee, NIH

HONORS: 1986-1988 Editor, Endocrinology
1986 Society for the Study of Reproduction Research Award
1987 Oppenheimer Award, Endocrine Society
1988 President's Achievement Award, Society for Gynecologic Investigation
1992 Director, Ninth Ovarian Workshop
1993 Transatlantic Medal, British Endocrine Society
1997 Merit Award, NICHD, NIH

SELECTED PUBLICATIONS (from last 3 years, total 330):

1. Leo CP, Hsu SY, Chun SY, Bae HW, Hsueh AJW 1999 Characterization of the antiapoptotic Bcl-2 family member Myeloid cell leukemia-1 (Mcl-1) and the stimulation of its message by gonadotropins in the rat ovary. Endocrinology (Dec.)
2. Hayashi M, McGee EA, Min G, Klein C, Ross U, van Duin M, Hsueh AJW 1999 Recombinant growth differentiation factor-9 (GDF-9) enhances growth and differentiation of cultured preantral follicles. Endocrinology 140:1236-44.
3. Chun SY, McGee EA, Hsu SY, Minami S, LaPolt PS, Yao HH, Bahr JM, Gougeon A, Schomberg DW, Hsueh AJW 1999 Restricted Expression of WT1 Messenger RNA in Immature Ovarian Follicles: Uniformity in Mammalian and Avian Species and Maintenance during Reproductive Senescence. Biol Reprod 60: 365-373.
4. Hsu SY, Liang SG and Hsueh AJW 1998 Characterization of two LGR genes homologous to gonadotropin and thyrotropin receptors with extracellular leucine-rich repeats and a G protein-coupled, seven-transmembrane region. Molecular Endocrinol 12:1830-1845.
5. Leo CP, Hsu SY, McGee E, Salanova M, Hsueh AJW 1998 DEFT, a novel death effector domain-containing molecule with predominant testicular expression. Endocrinology 139: 4839-4848.

6. Hsu SY and Hsueh AJW 1998 A splicing variant of the Bcl-2 member Bok with a truncated BH3 domain induces apoptosis without dimerization with antiapoptotic Bcl-2 proteins. J Biol Chem 273: 30139-30146.
7. Wiersma A, Hirsch B, Tsafriri A, Hansen R, Vander Kant M, Kloosterboer HJ, Conti M, Hsueh AJW 1998 Phosphodiesterase 3 inhibitors suppress oocyte maturation and consequent pregnancy without affecting ovulation and cyclicity in rodents. J. Clin. Invest. 102: 532-537
8. Hsu SY, Lin P, Hsueh AJW 1998 BOD is an ovarian BH3 domain-containing pro-apoptotic Bcl-2 protein capable of dimerization with diverse anti-apoptotic Bcl-2 members. Mol Endocrinol 12:1432-1440
9. Hsu SY, Kaipia A, McGee E, Lomeli M and Hsueh AJW 1997 Bok is a pro-apoptotic Bcl-2 protein with restricted expression in reproductive tissues and hetero-dimerizes with selective anti-apoptotic Bcl-2 proteins. Proc. Natl. Acad. Sci. USA 94: 12401-12406.
10. Osuga Y, Hayashi M, Kudo M, Conti M, Kobilka B, Hsueh AJW 1997 Co-expression of defective luteinizing hormone receptor fragments partially reconstitutes ligand-induced signal generation. J Biol Chem 272:25006-25012.
11. Osuga Y, Kudo M, Kaipia A, Kobilka B, Hsueh AJW 1997 Derivation of functional antagonists using N-terminal extracellular domain of gonadotropin and thyrotropin receptors. Mol Endocrinol 11:1659-1668.
10. McGee E, Perlas E, LaPolt PS, Hsueh AJW 1997 Follicle-stimulating hormone enhances the development of preantral follicles in juvenile rats. Biol Reprod 57:990-998
11. Hsu SY, Kaipia A, Zhu L, Hsueh AJW 1997 Interference of BAD-induced apoptosis in mammalian cells by proteins of the 14-3-3 family and P11. Mol Endocrinol 11:1858-1867
12. Kaipia A, Hsu SY Hsueh AJW 1997 Expression and function of a pro-apoptotic Bcl-2 family member BAD in the rat ovary. Endocrinology 138:5497-5504
13. McGee E, Spears N, Minami S, Hsu SY, Chun SY, Billig H, Hsueh AJW 1997 Preantral ovarian follicles in serum-free culture: suppression of apoptosis following activation of the cGMP pathway and stimulation of growth and differentiation by FSH. Endocrinology 138:2417-2424
14. Eisenhauer KM, Gerstein RM, Chiu CP, Conti M, Hsueh AJW 1997 Telomerase activity in mammalian female and male germ cells undergoing meiosis and in early embryos. Biol Reprod 56:1120-1125
15 Garcia-Campayo V, Sato A, Hirsch B, Sugahara T, Muyan M, Hsueh AJW, Boime I 1997 Design of stable biologically active recombinant lutropin analogs. Nature Biotech 15:663-667
16. Kaipia A, Chun SY, Eisenhauer K, Hsueh AJW 1996 Tumor necrosis factor-alpha and its second messenger, ceramide, stimulate apoptosis in cultured ovarian follicles. Endocrinology 137:4864-4870
17. Hsu SY, Lai RJ, Finegold M, Hsueh AJW 1996 Targeted overexpression of Bcl-2 in ovaries of transgenic mice leads to decreased follicle apoptosis, enhances folliculogenesis and increased germ cell tumorigenesis. Endocrinology 137:4837-4843
18. Hirsch B, Kudo M, Naro F, Conti M, Hsueh AJW 1996 The C-terminal third of the human LH receptor is important for inositol phosphate release: analysis using chimeric human LH/FSH receptors. Mol Endocrinol 10: 1127-1137
19. Kudo M, Osuga Y, Kobilka BK, Hsueh AJW 1996 Transmembrane regions V and VI of the human luteinizing hormone receptor are required for constitutive activation by a mutation in the third intracellular loop. J Biol Chem 271:22470-22478
20. Laue LL, Wu SM, Kudo M, Bourdony CJ, Cutler GB, Hsueh AJW, Chan WY 1996 Compound heterozygous mutations of the LH receptor gene in Leydig cell hypoplasia. Mol Endocrinol 10:987-997
21. Tsafriri A, Chun SY, Zhang R, Hsueh AJW, Conti M 1996 Oocyte maturation involves compartmentalization and opposing changes of cAMP levels in follicular somatic and germ cells: studies using selective phosphodiesterase inhibitors. Develop Biol 178:393-402
22. Chun SY, Eisenhauer KM, Minami S, Billig H, Perlas E, Hsueh AJW 1996 Hormonal regulation of apoptosis in early antral follicles: FSH as a major survival factor. Endocrinology 137:1447-1456